

I. AMENDMENTS TO THE CLAIMS

Please enter the amendments to claims 7, 9, and 11, as shown below.

Please enter new claims 28-46, as shown below.

1.-6. (Canceled)

7. (Currently Amended) A method of detecting specific lysis of a target cell in a plurality of different target cells, comprising:

a) contacting a lytic agent with:

i) a first target cell labeled with a first plasma membrane-labeling fluorescent dye and a cytosol-labeling fluorescent dye; and

ii) at least a second target cell labeled with a second plasma membrane-labeling fluorescent dye and the cytosol-labeling fluorescent dye;

b) ~~determining~~ detecting a reduction in the amount of fluorescent cytosol-labeling dye ~~remaining~~ in the first and at least the second target cell, wherein a reduction in the amount of the cytosol-labeling fluorescent dye in any of said target cells indicates that ~~the~~ a target cell is has been lysed by the lytic agent; and

c) ~~relating determining the plasma membrane-labeling fluorescent dye in the unlysed target cell to~~ the identity of the unlysed target cell, by detecting the fluorescent plasma membrane labeling dye in the unlysed target cell, and thereby detecting the specific lysis of a target cell.

8. (Original) The method of claim 7, wherein at least one of said plurality of target cells is a control target cell.

9. (Currently amended) The method of claim 7, wherein the first target cell and the second target cell are donor target cells ~~at least two different target cells comprise cells~~ from different potential tissue or organ donors, and the lytic agent is a cell from a prospective recipient of a tissue or organ.

10. (Original) The method of claim 9, wherein the donor target cells and the lytic cell are peripheral blood mononuclear cells.

11. (Currently Amended) A method of detecting specific lysis of a target cell in a plurality of different target cells, comprising:

- a) contacting a lytic agent with
 - i) a first target cell labeled with a first cytosol-labeling fluorescent dye and a plasma membrane-labeling fluorescent dye; and
 - ii) at least a second target cell labeled with a second cytosol-labeling fluorescent dye and the plasma membrane-labeling fluorescent dye;
- b) ~~determining~~ detecting a reduction in the amount of fluorescent cytosol-labeling dye remaining in the first and at least the second target cell, wherein a reduction in the amount of the cytosol-labeling fluorescent dye in any of said target cells indicates that ~~the a~~ target cell is has been lysed by the lytic agent; and
- c) ~~relating determining the cytosol-labeling fluorescent dye in the unlysed target cell to~~ the identity of the unlysed target cell, by detecting the fluorescent cytosol-labeling dye in the unlysed target cell, and thereby detecting the specific lysis of a target cell.

12.-27. (Canceled)

28. (New) The method of claim 7, wherein said first and said second plasma membrane-labeling fluorescent dyes are lipid-associated fluorescent dyes, and wherein said cytosol-labeling fluorescent dye is a fluorescent dye that labels proteins in the cytosol.

29. (New) The method of claim 7, wherein the lytic agent is a cell having lytic activity toward one of the target cells.

30. (New) The method of claim 29, wherein the cell having lytic activity toward one of the target cells is an antigen-specific CD8⁺ T lymphocyte, and the target cell displays the antigen in an MHC Class I molecule on its cell surface.

31. (New) The method of claim 7, wherein the lytic agent is an antibody specific for a cell surface marker on the target cell.

32. (New) The method of claim 7, wherein the emission of the first plasma membrane-labeling fluorescent dye differs from the emission of the second plasma membrane-labeling fluorescent dye by at least about 10 nm.
33. (New) The method of claim 28, wherein said lipid-associated fluorescent dyes are selected from PKH-26, PKH-67, and a long chain dialkylcarbocyanine.
34. (New) The method of claim 28, wherein the protein-labeling cytosol dye is selected from 5-(-6)-carboxyfluorescein, 5-(-6)(((4-chloromethyl)benzoyl) amino) tetramethylrhodamine), 7-amino-4-chloromethylcoumarin, and a SNARF® fluorescent dye.
35. (New) The method of claim 7, wherein said detecting of a reduction in the amount of cytosol-labeling fluorescent dye is carried out using flow cytometry.
36. (New) The method of claim 11, wherein at least one of said plurality of target cells is a control target cell.
37. (New) The method of claim 11, wherein the first target cell and the second target cell are donor target cells from different potential tissue or organ donors, and the lytic agent is a cell from a prospective recipient of a tissue or organ.
38. (New) The method of claim 37, wherein the donor target cells and the lytic cell are peripheral blood mononuclear cells.
39. (New) The method of claim 11, wherein said plasma membrane-labeling fluorescent dye is a lipid-associated fluorescent dye, and wherein said first and said second cytosol-labeling fluorescent dyes are fluorescent dyes that label proteins in the cytosol.
40. (New) The method of claim 11, wherein the lytic agent is a cell having lytic activity toward one of the target cells.

41. (New) The method of claim 40, wherein the cell having lytic activity toward one of the target cells is an antigen-specific CD8⁺ T lymphocyte, and the target cell displays the antigen in an MHC Class I molecule on its cell surface.
42. (New) The method of claim 11, wherein the lytic agent is an antibody specific for a cell surface marker on the target cell.
43. (New) The method of claim 11, wherein the emission of the first cytosol-labeling fluorescent dye differs from the emission of the second cytosol-labeling fluorescent dye by at least about 10 nm.
44. (New) The method of claim 39, wherein said lipid-associated fluorescent dyes are selected from PKH-26, PKH-67, and a long chain dialkylcarbocyanine.
45. (New) The method of claim 39, wherein the protein-labeling cytosol dye is selected from 5-(-6)-carboxyfluorescein, 5-(-6)((4-chloromethyl)benzoyl amino) tetramethylrhodamine, 7-amino-4-chloromethylcoumarin, and a SNARF® fluorescent dye.
46. (New) The method of claim 11, wherein said detecting of a reduction in the amount of cytosol-labeling fluorescent dye is carried out using flow cytometry.